Review Article

Is the Future of Energy Rotten? Novel Perspective on Tri-Phase Fermentation and the Food Waste Paradox

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Abstract
Annually, a staggering 1.3 billion tons of edible food are wasted globally, representing not only a substantial economic loss but also a squandered opportunity for sustainable energy production. While anaerobic digestion offers a potential pathway for valorizing this waste, its limitations in feedstock conversion efficiency and substrate versatility necessitate the exploration of innovative alternatives. This comprehensive perspective review elucidates the transformative potential of tri-phase fermentation (TPF), a groundbreaking approach that represents a paradigm shift in waste valorization by synergistically integrating solid-state fermentation (SSF), submerged fermentation (LF), and gas fermentation (GF) to derive bioethanol from food waste. This study highlights the successful integration of these three phases within the TPF framework, demonstrating effective carbohydrate breakdown in SSF, significant ethanol production in LF, and valuable product generation from syngas in GF. By harnessing the metabolic capabilities of diverse microorganisms and leveraging emerging technologies, TPF offers a holistic solution, effectively converting both the primary food waste and its residual byproducts into valuable bioethanol. This review critically examines the fundamental principles, comparative advantages, and inherent challenges associated with each fermentation phase, while also elucidating their potential for synergistic integration within the TPF framework. Furthermore, the technological and economic hurdles inherent to TPF are addressed, emphasizing the need for further research in strain engineering, process optimization, and downstream processing to enhance its commercial viability. This review accentuates and provides a comprehensive perspective on the urgent need for further research and development to fully unlock the transformative potential of TPF and promote a circular bioeconomy by converting food waste into valuable bioethanol, addressing both waste and energy challenges.

Keywords: Bioethanol, Circular bioeconomy, Food waste, Sustainable energy, Tri-phase fermentation

1 Introduction

Food waste (FW), representing a staggering 1.3 billion tons of edible food wasted annually, presents a pressing global challenge with significant economic and environmental ramifications [1]. Simultaneously, the escalating global energy demand calls for a shift towards sustainable energy sources to mitigate the adverse environmental impact of fossil fuels [2]. The heavy reliance on fossil fuels has led to a concerning rise in CO_2 levels and significant carbon emissions, contributing to climate change and air pollution [3].

FW, with its biodegradability and high organic content, offers a promising avenue for sustainable energy generation [4]. Anaerobic digestion, a common method for converting FW into biogas, offers one such

sustainable solution [5]. However, its limitations—including incomplete feedstock conversion, inability to utilize certain waste types, and challenges in efficiency, scalability, and product contamination—hinder its widespread adoption [6].

Fermentation, a versatile biotechnological process, employs microorganisms to convert organic substrates into useful products [7]. While solid-state fermentation (SSF) and submerged (liquid) fermentation (LF) offer distinct advantages [8]–[10], an emerging technology—gas fermentation (GF)—utilizes gases like carbon monoxide and hydrogen to produce bioethanol and other valuable products [11]–[13]. Tri-phase fermentation (TPF), a novel approach coined in this context, integrates solid, liquid, and GF in a holistic process. It aims to not only valorize primary waste streams, such as FW but also to utilize waste products from each fermentation phase, further promoting a circular economy. This synergistic approach presents significant potential to enhance sustainable bioethanol production and resource recovery from what was once considered mere waste.

While TPF shows promise in addressing the dual challenges of FW management and sustainable energy production, its relative novelty and the current scarcity of comprehensive research necessitate a thorough assessment of its current state, potential, and challenges to guide future research and development. This review aims to bridge this knowledge gap by delving into the technology and practical applications of various fermentation techniques, with a particular focus on the potential of TPF for promoting a circular economy and maximizing resource efficiency. Furthermore, given the recent advancements in bioprocessing and the escalating urgency to address FW and energy concerns, this review is particularly timely in exploring potential future directions and research areas to further advance these technologies. Table 1 provides a comparative overview of the literature and technologies covered, highlighting the novelty and contribution of this study, with categories including FW, SSF, LF, GF, supercritical water gasification (SCWG), bioethanol, techno-economic analysis (TEA), life-cycle assessment (LCA) and TPF.

Table 1: Comparative overview of literature and technologies for food waste valorization and bioethanol production.

FW	SSF	LF	GF	SCWG	Bioethanol	TEA	LCA	TPF	Reference
X	X	X	X	X	√	X	X	X	[14]
X	X	X	X	X	√	√	√	X	[15]
√	X	X	X	X	√	X	√	X	[16]
√	X	X	X	X	√	X	X	X	[17]
√	√	√	X	X	√	X	X	X	[18]
√	√	X	X	X	√	X	X	X	[19]
X	X	√	√	X	√	√	X	X	[20]
√	√	√	√	√	√	√	√	√	This study

2 Food Waste

The escalating global production of waste, now reaching billions of tons of biological waste annually, presents a significant environmental and resource management challenge [21], [22]. While the issue of food waste is widely recognized, other waste streams are also experiencing a concerning upward trend. Agricultural waste, including crop residues like rice husks and corn stover, accounts for approximately 154.5 billion USD per year [23]. Similarly, household waste, encompassing materials such as paper, plastics, and yard waste, contributes another 750 billion USD annually [24].

Despite the challenges posed by these diverse waste streams, the food production chain generates a particularly large amount of FW, posing a more immediate threat to both human health and the environment [25]. The substantial carbon content in FW contributes significantly to greenhouse gas emissions, exacerbating global warming concerns. Moreover, FW carries a hefty economic price tag, with estimates suggesting that one-third of produced food lost or wasted globally costs a staggering 2.6 trillion USD and releases 4.4 Gt CO_2 eq of greenhouse gasses [26].

Due to the sheer volume and detrimental impacts of FW, particularly concerning greenhouse gas emissions, it remains a primary concern, thus necessitating and justifying the focus of this study. To effectively harness this potential and develop sustainable solutions, this section explores the complex nature of FW, its valorization potential, and its energy recovery potential, culminating in the introduction of the novel concept of TPF as a promising avenue for bioethanol production.

2.1 *The global food waste challenge and its characteristics*

The Food and Agriculture Organization of the United Nations (2011) reports that per capita food loss varies considerably by region [27]. Estimates range from 280–300 kg/year in Europe and North America to a lower 120–170 kg/year in Sub-Saharan Africa and South/Southeast Asia. The primary crops wasted also differ regionally [28]. Wheat is prevalent in medium- and high-income countries, while rice is more commonly wasted in low-income regions [29]. In medium- and high-income countries, potato (or sweet potato in China) is another major source of FW [30]. Cassava is commonly wasted in Sub-Saharan Africa and Latin America, sunflower seed and rapeseed in Europe [31], soybean in North America, Oceania, and Industrialized Asia, and groundnut in Sub-Saharan Africa [32]. These losses are evident in Figure 1 [27].

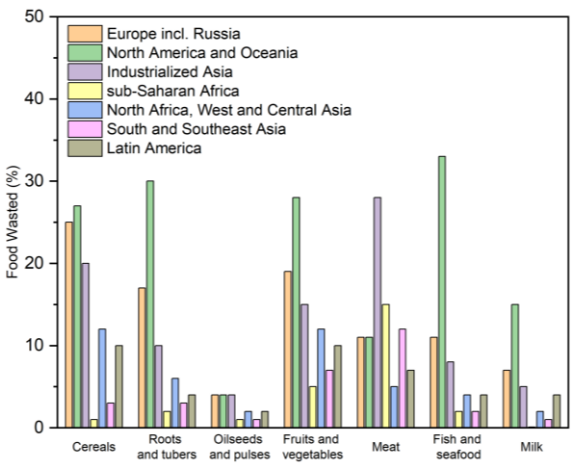

Figure 1: Regional food loss and waste by commodity [27].

The substantial global loss and waste of food highlight the urgent need for improved practices and infrastructure throughout the food supply chain [33]. This pervasive issue affects both industrialized and developing regions, albeit with distinct patterns [34].

In industrialized regions, such as Europe, North America, and Oceania, consumer-level waste is high, particularly for perishable items like fruits, vegetables, and meat. This trend suggests that consumer behavior and practices play a significant role in FW generation in these areas [35]. In North America and Oceania, a staggering 33% of fish and seafood is wasted, highlighting inefficiencies in the supply chain and consumer behavior [36].

In contrast, developing regions, especially sub-Saharan Africa, experience substantial losses primarily during the early and middle stages of the food supply chain, including production, post-harvest handling, and processing [37]. This pattern is evidenced by the minimal waste of commodities like milk and cereals in sub-Saharan Africa, suggesting potential areas for improvement in other regions [38]. These losses in developing regions point to infrastructural challenges, inadequate storage facilities, and technical limitations as key factors [39].

However, amidst this challenge lies a valuable opportunity. FW can be valorized offering a path towards resource recovery and sustainable energy production [40]. To effectively harness this potential, a thorough understanding of the diverse characteristics of FW is crucial [1], and Figure 2 further details the nutritional composition of the most commonly wasted food and crops [41]–[47].

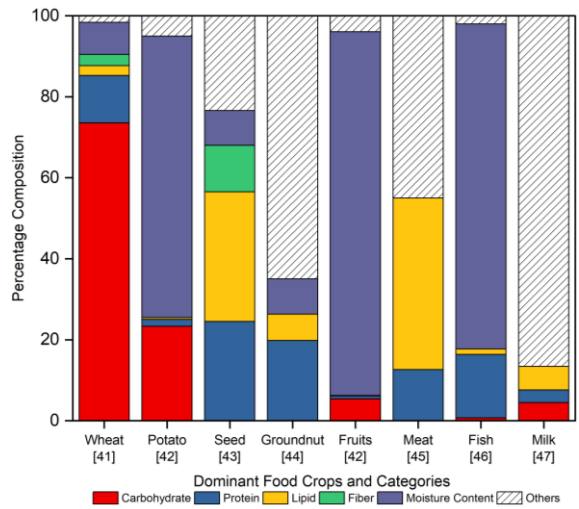

Figure 2: Macronutrient profile of dominant food crops and categories (pre-consumption) [41]–[47].

Figure 2 presents a detailed analysis of the nutritional composition of various food groups, emphasizing their diverse macronutrient profiles, which ultimately influence the composition of FW [48]. Cereals, such as wheat, are primarily characterized by their high carbohydrate content (73.56%), serving as a primary source of energy [49]. In contrast, oilseeds and pulses, represented by sunflower seeds and groundnuts, respectively, are distinguished by their high lipid (32% and 6.51%, respectively) and protein content (24.5% and 19.82%, respectively) [43], [44]. Roots and tubers, such as

potatoes, also contribute significantly to carbohydrate intake (23.32%) but possess a higher moisture content (69.51%) compared to cereals [42], [50]. Fruits, such as oranges, are predominantly composed of water (89.8%) and carbohydrates (5.37%), with lower amounts of protein and lipids [42, 51]. Meat and fish serve as excellent sources of protein, with meat also containing a substantial amount of lipids [45]. Milk offers a balanced mix of macronutrients, encompassing carbohydrates (4.5%), proteins (3.1%), and lipids (5.8%) [47].

The composition of generated FW is directly influenced by the prevalence of different food items within each commodity group [52]. Figure 2 highlights the diverse nutritional profiles of these food items, which in turn impact the characteristics of the resulting waste, as further elaborated in Figure 3 [53]–[63].

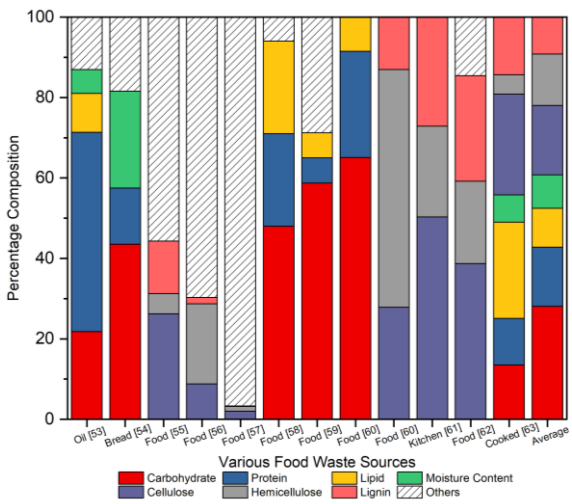

Figure 3: Nutritional and lignocellulosic content of various food waste [53]–[63].

Figure 3 unveils the hidden potential within FW, exhibiting its complex composition that encompasses both nutritional and lignocellulosic properties, ripe for valorization. Carbohydrates emerge as the predominant component, averaging approximately 41.87%, followed by protein at 21.9% and lipids at 14.48%. The substantial presence of carbohydrates and protein underscores the potential of FW as a valuable feedstock for nutrient and energy recovery [64]. This analysis further reveals significant variability in these values, ranging from as low as 6.25% for protein to as high as 49.56% for carbohydrates, highlighting the diverse nature of FW and the need for adaptable valorization strategies. Protein content determination has involved Kjeldahl and Dumas methods, while lipid content has been assessed via Soxhlet extraction [53], [59], [65]. The moisture content of FW, averaging around 12.4%, can exhibit substantial variation, reaching up to 24.1% in some instances [54]. This amount of moisture content necessitates pretreatment procedures such as drying or dewatering, tailored to the specific valorization pathway [5]. Furthermore, based on the diverse composition of FW, different preparation methods might be more suitable for different waste types. On carbohydrate-rich fruit and vegetable waste, a simple drying and grinding approach is sufficient for analysis, whereas biomass with significant lignocellulosic content necessitates complex or sequential procedures [53]–[63].

Furthermore, Figure 3 elucidates the lignocellulosic composition of FW. Cellulose is the most abundant component, averaging approximately 25.72%, followed by hemicellulose and lignin at 19.05% and 13.74%, respectively. The presence of these lignocellulosic components, albeit variable, suggests the potential for utilizing FW as a feedstock for biofuel production or the extraction of valuable chemicals through processes such as hydrolysis and fermentation [66], [67].

A comparative analysis of the nutritional compositions in Figure 2 (pre-consumption) and Figure 3 (post-consumption) reveals both similarities and key distinctions. The average carbohydrate content of FW aligns with the range observed for wheat (cereals) and potato (roots and tubers), suggesting a significant portion of the waste originates from these food groups [68]. Similarly, the protein content of FW is comparable to that of sunflower seeds and groundnuts, indicating a potential origin from oilseeds and pulses [69]. The lipid content can be attributed to meat and fish sources [70]. Beyond its nutritional and lignocellulosic composition, FW can also be characterized by its wastewater form, as detailed in Table 2.

Table 2: Comparative overview of physicochemical properties for food waste valorization.

Source	TS (%)	VS (%)	VS/TS (%)	TSS (mg/L)	COD (mg/L)	BOD_5 (mg/L)	TN (mg/L)	TP (mg/L)	pH	Ref.
FWW and oil	ND	ND	ND	239	335	177	38	3.7	7.4	[71]
FWW	15.9	15.0	94.1	ND	14494	ND	ND	ND	5.2	[72]
FWW	7.6	7.2	95	ND	129.8	ND	ND	ND	4.2	[73]
FWW	ND	ND	ND	22500	90000	51050	1650	255	3.8	[74]
FWW and sludge	ND	ND	94.52	1011.4	1252	ND	1.6	ND	5.5	[75]
FWW and corn	20.57	19.89	96.7	ND	335125	ND	ND	ND	5.34	[76]
FWW and garden	9.11	8.53	93.6	ND	103687	ND	ND	ND	4.3	[58]
FWW and pulp	89000 [ab]	83600 [ab]	ND	ND	66000	ND	1.9	26.0	5.2	[77]
FWW	86230[ab]	81373[ab]	94	ND	65715	ND	2.7[ac]	1.7 [ac]	4.92	[60]
Mean±SD	13.30 ±5.24	12.66 ±5.11	94.65 ±1.01	7916.8± 10316.7	75193.09 ±99532.23	25613.5± 25436.5	422.875± 708.64	94.9± 113.57	5.10± 0.98	

ND – Not determined, [a] – not included in mean±SD, [b] – mg/L, [c] – g/kg

Food wastewater (FWW) typically exhibits high levels of organic matter, nutrients, and suspended solids. However, its specific composition can vary considerably depending on its source and the nature of the FW itself [78]. Total Solids (TS) content ranges from 7.6% to 20.57%, with an average of 13.3%. Of these solids, a substantial proportion (average of 94.65%) are Volatile Solids (VS), indicating a high content of readily biodegradable organic matter [79, 80]. The high organic load is further emphasized by the Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD_5) values, which average 75,193 mg/L and 25,613 mg/L, respectively, but can reach as high as 335,125 mg/L and 51,050 mg/L [74], [76].

Additionally, FWW contains significant levels of nutrients [81], with average Total Nitrogen (TN) and Total Phosphorus (TP) levels of 422.87 mg/L and 94.9 mg/L, respectively. The average pH of 5.1 indicates a slightly acidic environment. These characteristics, particularly the high organic content and nutrient levels, make FWW a promising candidate for energy recovery processes [82], where the organic matter can be converted into a renewable energy source [7].

2.2 *Valorization of food waste*

Recognizing the diverse characteristics and challenges associated with FW, as detailed in the preceding section, innovative approaches are being developed to transform this environmental burden into a valuable resource [83]. FW valorization encompasses a range of these approaches, aiming to convert FW into renewable energy, valuable nutrients, and a spectrum of value-added products [84], [85]. Central to this concept is the idea of food value addition, where discarded food materials are reused or repurposed to create new products with enhanced economic or functional value. Figure 4 provides a visual overview of the various pathways for FW valorization, highlighting the potential to recover energy, nutrients, and other valuable resources from this underutilized stream.

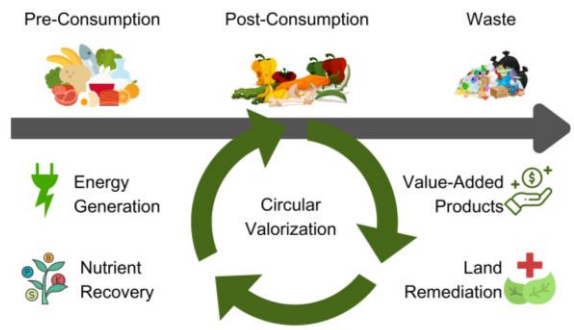

Figure 4: Overview of food waste valorization pathways.

2.2.1 *Energy generation*

FW represents a significant untapped resource for renewable energy production. Through common processes such as anaerobic digestion, biogas rich in methane can be generated [6], [86]. This biogas can be utilized for various purposes, including heat and electricity generation, or even upgraded to biomethane for injection into the natural gas grid. Anaerobic digestion offers the advantage of being a well-

established technology with the potential for large-scale implementation. However, challenges such as the need for pretreatment to enhance biogas yield, the potential for process instability due to feedstock variability, and the presence of contaminants in the biogas can limit its widespread adoption [87], [88].

Fermentation processes present another pathway to convert FW into biofuels, notably bioethanol [89]. This versatile biofuel can be blended with gasoline to reduce fossil fuel consumption or used directly in fuel cells for electricity generation. The choice of fermentation method depends on the specific characteristics of the FW [90], [91], with options such as LF, SSF, and emerging technologies like GF.

2.2.2 Nutrient recovery

Beyond energy generation, recovering valuable nutrients from FW not only reduces its environmental impact but also creates opportunities for sustainable resource utilization [92]. Composting, a natural biological process, transforms FW into nutrient-rich compost. This compost serves as a valuable soil amendment, providing essential macro and micronutrients like nitrogen, phosphorus, and potassium to enhance soil fertility, improve water retention, and promote plant growth [93].

Insect farming offers an innovative approach to nutrient recovery, utilizing FW as a feedstock for insect larvae, such as black soldier flies [94]. These larvae efficiently convert FW into protein-rich biomass, which can be used as a sustainable and nutritious alternative to conventional animal feed. The potential benefits of insect farming include reduced greenhouse gas emissions, lower land and water requirements compared to traditional livestock production, and the potential for human consumption of insect-based protein [95].

Furthermore, nutrient-rich leachate, a liquid byproduct generated during the decomposition of FW, can be extracted and harnessed in hydroponic systems to cultivate crops. This approach allows for efficient nutrient recycling and reduces the reliance on synthetic fertilizers [96].

2.2.3 Value-added products

Beyond energy and nutrient recovery, FW can serve as a feedstock for the production of diverse value-added products [40]. This represents a key aspect of food value addition. Bioplastics, synthesized from FW-derived sugars through microbial fermentation or chemical conversion, offer a sustainable alternative to conventional petroleum-based plastics. These bioplastics can be biodegradable or compostable, helping to mitigate plastic pollution and reduce greenhouse gas emissions. Examples include polyhydroxyalkanoates (PHAs) and polylactic acid (PLA), which can be used in packaging, agriculture, and other sectors [97].

The biochemical potential of FW is also considerable [1]. Through fermentation and other bioprocesses, FW can be converted into a range of valuable chemicals, including organic acids, enzymes, and biopolymers. These biochemicals have applications in various industries, such as food, pharmaceuticals, cosmetics, and textiles. Specific examples include the production of succinic acid for use in bioplastics, citric acid as a food additive, and enzymes like proteases and lipases for various industrial applications [25].

In addition to bioplastics and biochemicals, processed FW can be incorporated into animal feed formulations, providing a sustainable and cost-effective source of nutrients. This approach helps to reduce the reliance on traditional feed ingredients and contributes to a more circular food system [98]. Furthermore, FW can be processed into novel food ingredients for human consumption, such as protein-rich powders or functional fibers, after appropriate safety and quality assessments.

2.2.4 Land remediation

The application of FW in land remediation offers a promising avenue for sustainable waste management and environmental restoration. Compost derived from FW, rich in organic matter and beneficial microorganisms, can serve as a valuable amendment for contaminated soils [98]. Its application can stimulate the activity of indigenous microbial communities, enhancing their capacity to degrade various pollutants, including hydrocarbons, heavy metals, and pesticides. The organic matter in compost also improves soil structure, water retention, and nutrient availability, facilitating the re-establishment of vegetation and promoting the overall ecological recovery of degraded land [99].

2.3 ***Energy recovery potential of food waste and wastewater***

The energy recovery potential of a material is a key factor in determining its suitability for sustainable

waste management practices [5]. Carbohydrates, due to their relatively high biodegradability and energy content, are a primary benchmark for evaluating this potential [6]. Cellulose and hemicellulose, complex carbohydrates commonly found in plant-based materials, represent a substantial source of energy that can be harnessed through various conversion technologies [100]. In wastewater, VS and COD are important indicators for assessing energy recovery potential, as they signify the presence of readily degradable organic matter suitable for energy generation. Both FW and FWW exhibit these characteristics, making them promising candidates for energy recovery initiatives [80]. Figure 5 further showcases these characteristics.

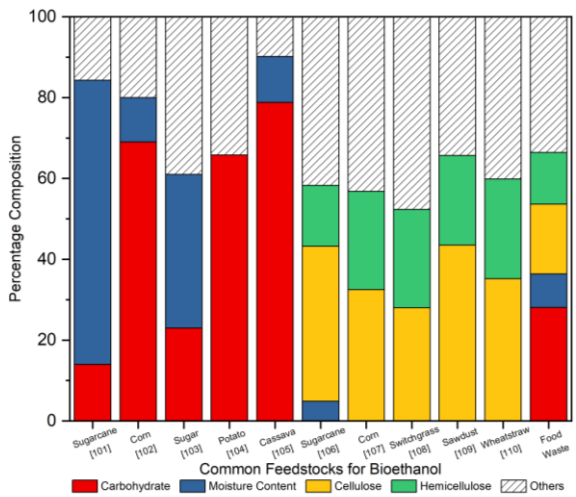

Figure 5: Composition of common feedstocks for bioethanol production [101]–[110].

FW, when assessed based on key compositional parameters presented in Figure 5, exhibits promising potential for bioethanol production, especially when compared to conventional feedstocks [111]. While the average carbohydrate content of food waste (41.87%) may not be as high as that of traditional sugar-rich crops like sugar beet (64%), it still represents a substantial source of fermentable sugars [101], [103]. The mean cellulose content of FW (25.72%) is comparable to sources like switchgrass [108], which is also being explored as a potential feedstock for bioethanol production [112]. Furthermore, the hemicellulose fraction in FW (19.05%) is comparable to most materials listed, including dedicated energy crops like switchgrass (24.32%) and corn stover (24.3%). Hemicellulose, although often overlooked, is a valuable source of fermentable sugars, thereby enhancing the overall bioethanol yield potential of FW [6], [113]. Additionally, the mean moisture content of FW (12.4%), while higher than lignocellulosic feedstocks, is considerably lower than starchy sources like sugarcane (70.3%) and sugar beet (64%) [104], [105]. This lower moisture content could potentially reduce energy expenditure during the pretreatment and fermentation stages [25], [114]. The combination of readily available carbohydrates and substantial lignocellulosic content in FW, coupled with its relatively low moisture content, makes it an attractive feedstock for TPF [6], [115]. Table 3 further extrapolates the attractiveness of FW on its wastewater form for energy generation.

Table 3: Primary physicochemical characteristics of various wastewater for biofuel production.

	COD (mg/L)	VS/TS (%)	pH	Ref.
Municipal	406	72	7.2	[116]
Municipal	364.4	94.7	7.41	[117]
Municipal	413.5	91.9	ND	[118]
Industrial	377.7	ND	7.76	[119]
Industrial	32300	64.2	5.5	[120]
Industrial	22636.83	82.1	6.73	[121]
Agricultural	360	77.2	7.02	[122]
Agricultural	2011.1	ND	6.3	[123]
Agricultural	1940	ND	6.95	[124]
FW (mean)	75193.09	94.65	5.10	

In addition to solid FW, the liquid fraction, or FWW, also holds significant potential for energy production, particularly when compared to other wastewater types [5], [121], [125], as highlighted in Table 3. Its high mean COD of 75,193.09 mg/L, with values ranging up to 335125 mg/L [73], significantly surpasses that of municipal (≤413.5 mg/L) [118], industrial (≤32,300 mg/L) [120], and agricultural wastewater (≤2011.1 mg/L) [123], indicating a substantially higher concentration of organic matter available for anaerobic digestion or other bioenergy conversion processes [7], [126]. Moreover, the VS/TS ratio of 94.65% in FWW signifies that the majority of its total solids are readily biodegradable, offering a distinct advantage over other wastewater sources [6], [127]. The high organic content and readily biodegradable nature of FWW, as evidenced by its high COD and VS/TS ratio, make it a particularly attractive feedstock for bioethanol production through TPF [5], [30], [40], [128]. The slightly acidic pH (average of 5.10) might necessitate pretreatment steps to optimize fermentation processes [7], [89].

2.4 *Tri-phase fermentation of food waste for sustainable bioethanol production*

While FW shows promise as a feedstock for bioethanol, its complex composition, rich in lignocellulosic material, poses challenges for direct fermentation [129], [130]. Pretreatment is necessary to unlock the carbohydrates trapped within this recalcitrant material [131]–[133]. Additionally, while the high volatile solids content in FW is beneficial for anaerobic digestion, the accompanying high COD presents limitations, as anaerobic digestion cannot fully process the complex carbons present [134].

Emerging pretreatment technologies offer potential solutions to these challenges. Enzymatic hydrolysis (EH) utilizes enzymes to break down complex carbohydrates into fermentable sugars [135], [136]. This approach is highly specific and efficient, but the high cost of enzymes and long reaction times can be limiting [137]. SSF, on the other hand, cultivates microorganisms directly on a solid substrate, enabling simultaneous saccharification and fermentation. SSF is cost-effective and environmentally friendly, but mass transfer limitations and slow reaction rates due to the solid nature of the substrate can be drawbacks [132].

Beyond the pretreatment of solid FW, innovative technologies are revolutionizing wastewater treatment [138]. SCWG, a gasification process, offers a potential alternative to anaerobic digestion for wastewater treatment and energy production. Operating at high temperatures and pressures, SCWO achieves rapid and complete oxidation of organic matter, even recalcitrant compounds [139]. This leads to high COD removal efficiency and the generation of syngas, a clean energy source composed of carbon monoxide and hydrogen. While syngas has applications in heat and power generation, its direct use for bioethanol production remains limited [140].

To utilize these sugars, several fermentation approaches can be employed. Separate Hydrolysis and Fermentation is a conventional approach where EH is performed as a separate step prior to fermentation. This allows for optimized conditions for both hydrolysis and fermentation but can lead to product inhibition during hydrolysis and requires separate reactors, increasing capital costs [141].

Another approach, Simultaneous Saccharification and Fermentation, combines EH and fermentation in a single step. SSF cultivates microorganisms directly on a solid substrate, enabling simultaneous saccharification and fermentation [142]. SSF is cost-effective and environmentally friendly, but mass transfer limitations and slow reaction rates due to the solid nature of the substrate can be drawbacks [132].

Building upon SSF, Simultaneous Saccharification and Co-Fermentation further integrate the fermentation of multiple sugars, typically hexoses and pentoses, using co-cultures or engineered microorganisms. This enhances overall sugar utilization and ethanol yields but requires careful selection and optimization of microbial strains [143].

Finally, Consolidated Bioprocessing represents the most integrated approach, where enzyme production, hydrolysis, and fermentation are all performed by a single microorganism or microbial consortium in one step [144]. CBP offers the potential for significant cost reductions but requires highly efficient microorganisms capable of performing all necessary functions [145].

The convergence of these emerging technologies paves the way for a novel and sustainable approach: TPF. This holistic process integrates solid, liquid, and GF to valorize FW and its byproducts across three distinct phases. In the solid phase, FW undergoes integrated EH and SSF, combining the efficiency of EH with the cost-effectiveness and environmental benefits of SSF [145]–[147]. The liquid phase involves the LF of the sugars released in the solid phase, further converting them into bioethanol [132], [147]. Lastly, the gas phase fermentation harnesses the syngas generated from SCWO treatment of FWW and solid residues, expanding the range of substrates for bioethanol production and maximizing resource recovery [139], [148], [149].

3 Enzymatic Hydrolysis (EH) and Solid-State Fermentation (SSF)

EH and SSF are emerging as powerful tools for the valorization of FW [147], [150]. EH employs specific enzymes to break down complex organic matter into simpler sugars [151], while SSF utilizes microorganisms to further degrade and convert these sugars into valuable products [152]. This combined approach offers a sustainable and efficient means to transform FW, a pressing environmental challenge [132].

3.1 *Enzymatic hydrolysis*

Hydrolysis is a fundamental process in the conversion of lignocellulosic biomass, involving the breakdown of complex molecules into simpler ones through the

addition of water [153]. This process is particularly crucial for transforming cellulose and hemicellulose, the main structural components of plant cell walls, into fermentable sugars that can be further utilized for biofuel and chemical production [154].

EH represents a specific type of hydrolysis that employs enzymes, such as cellulases and hemicellulases, to catalyze the breakdown of these complex carbohydrates [151]. This approach is favored over other hydrolysis methods, such as acid or alkaline hydrolysis, due to its inherent advantages of high specificity, mild reaction conditions, and environmental sustainability [155].

3.1.1 Principles of enzymatic hydrolysis

The EH process typically involves several key steps: 1) Enzyme adsorption onto the cellulose substrate, where the enzymes bind to the surface of the cellulose fibers. 2) Enzyme diffusion along the cellulose chains to locate suitable hydrolysis sites. 3) Cleavage of the β-1,4-glycosidic bonds that link the glucose units in cellulose, resulting in the release of cellobiose, cello-oligosaccharides, or glucose. 4) Diffusion of the released products away from the hydrolysis site, allowing the enzyme to continue the hydrolysis process or relocate to a new site [151], [154].

EH offers several distinct advantages. Its high specificity minimizes the generation of unwanted by-products, leading to a cleaner and more efficient process [131]. The mild reaction conditions employed in EH preserve the integrity of the released sugars and reduce energy consumption compared to harsher chemical hydrolysis methods [155]. Furthermore, the absence of harsh chemicals makes EH an environmentally friendly approach [151]. Additionally, the process offers a high degree of controllability, allowing for precise regulation of reaction rate and product distribution [156], [157].

However, EH also faces certain limitations. The production of enzymes can be costly, and challenges in enzyme recovery and reuse can further increase the overall process expenses [151]. Moreover, the reaction rate of EH is generally slower compared to chemical hydrolysis, potentially leading to longer processing times [99]. Finally, enzymes are sensitive to environmental factors such as pH and temperature, necessitating careful management of reaction conditions to maintain optimal enzyme activity and stability [152].

3.1.2 Factors affecting enzymatic hydrolysis of food waste

The utilization of FW as a substrate for EH, a process that employs enzymes to break down complex organic matter into simpler sugars suitable for biofuel or other value-added product conversion, has gained significant attention due to its potential to address waste management and energy challenges [138]. As detailed in Table 4, various factors, including enzyme type and dosage, pH, temperature, and pretreatment methods, can significantly influence the efficiency and yield of this hydrolysis process, ultimately impacting the subsequent conversion of FW [25]. This subsection explores these key factors and their impact on the hydrolysis of FW.

Table 4: Parameters and methods for enzymatic hydrolysis of food waste.

Source	Pretreatment	Enzyme Type	Enzyme Dosage	pH	Temperature (°C)	Hydrolysis Time (h)	Agitation Speed (rpm)	Subsequent Process	Ref.
FW	Size reduction	α-amylase	105 U/g FW	6.5	60	9.6	100	Bioethanol fermentation	[9]
FW	Drying	glucoamylase	60 U/g FW	4.5	55	4.4	100	Bioethanol fermentation	[9]
Restaurant	SSF (*Aspergillus oryzae*)	enzyme mixture	5% (w/w FW)	ND	60	24	100	Anaerobic digestion	[158]
FW	Ultrasound, Freeze-thaw, Hydrothermal, Drying	α-amylase, glucoamylase	36 U/g FW	4.5 – 6.5	55–60	12	100	ND	[159]
FW	Ultrasound	α-amylase	10-30 U/mL	5	70	12	ND	Bioethanol fermentation	[160]
Kitchen	Size reduction	α-amylase	150 U/g TS	5.5	50	16	150	Bioethanol fermentation	[161]
Kitchen	Autoclave	glucoamylase	150 U/g TS	4	60	7	150	Bioethanol fermentation	[161]
FW	SSF (*Aspergillus oryzae* and *Aspergillus niger*)	enzyme mixture	0.5% (w/v FW)	ND	60	48	150	Anaerobic digestion	[162]

ND – Not determined

As evident in Table 4, enzyme dosages vary widely, with optimal dosage depending on several factors, including enzyme type, activity, and the specific characteristics of the FW substrate [162]. Similarly, pH and temperature conditions require careful optimization to match the specific requirements of the enzymes used and the different stages of hydrolysis. Typically, pH ranges between 4 and 6.5, while temperature varies from 50°C to 70°C [160], [161]. Hydrolysis duration, influenced by factors such as enzyme dosage, temperature, and desired hydrolysis extent, can range from 7 to 48 h [57]. Agitation speed typically maintained between 100 and 150 rpm, ensures adequate mixing and mass transfer, promoting efficient enzyme-substrate interaction.

The choice of enzymes is critical and depends on the composition of the FW and the targeted end product. For starch-rich FW, α-amylase and glucoamylase are commonly employed due to their ability to efficiently break down starch into fermentable sugars [9], [157], [161]. When the goal is to enhance overall biodegradability for anaerobic digestion, a broader spectrum of enzymes, often sourced from fungal mash, is used to target diverse components, including proteins and cellulose [158].

The effectiveness of EH in FW valorization is evident in its ability to generate substantial reducing sugars, crucial for bioethanol production. The potential to achieve high sugar concentrations, often exceeding 100 g/L, has been reported, which is favorable for efficient bioethanol fermentation. Integrating pretreatment techniques, such as ultrasonication or hydrothermal treatment, can further enhance hydrolysis by increasing enzyme-substrate accessibility, leading to improved sugar yields and reduced processing times [159], [160].

Additionally, the utilization of *Aspergillus oryzae* and *Aspergillus niger* in SSF for in-situ fungal mash and microbial consortium production presents a cost-effective and sustainable alternative to commercial enzymes. This approach has the potential to enhance hydrolysis efficiency due to the synergistic action of multiple enzymes present in the fungal mash [158], [162].

3.2 *Solid-state fermentation*

SSF represents a distinct bioconversion process where microorganisms, predominantly fungi, are cultivated on a solid substrate in the near absence of free water [163]. This approach leverages the natural capability of fungi to degrade complex carbohydrates present in lignocellulosic biomass, such as FW, into simpler sugars [152]. These readily fermentable sugars then serve as substrates for further processing into biofuels, biochemicals, and other value-added products [131].

3.2.1 Principles of solid-state fermentation

The typical SSF process encompasses several key stages: 1) Inoculation of the pretreated substrate with the microbial culture. 2) Conducting the fermentation process under controlled conditions, including temperature, humidity, and aeration. During fermentation, the microorganisms secrete a variety of hydrolytic enzymes that break down the complex carbohydrates into simpler sugars. 3) Extraction and recovery of the desired products from the fermented solid matrix [132], [164].

SSF offers several notable advantages. Firstly, the versatility of SSF allows it to utilize a wide range of solid substrates, including agricultural residues, food processing byproducts, and even municipal solid waste [132]. This reduces the reliance on expensive and environmentally harmful chemicals often required in LF [152]. Secondly, SSF operates under relatively low moisture conditions. This minimizes the risk of contamination and reduces energy consumption associated with water handling and sterilization [163]. Finally, SSF can be conducted at high solid loadings, leading to increased product concentrations and reduced reactor volumes. This has the potential to improve process efficiency and economics [165].

Despite these advantages, SSF also presents certain challenges. The heterogeneous nature of the solid substrate can make it difficult to control the fermentation process, potentially impacting microorganism growth and metabolic activity [166]. Additionally, the slow mass transfer of nutrients and oxygen within the solid matrix can limit the overall fermentation rate. Finally, extracting and recovering the desired products from the solid matrix can be complex, often necessitating additional downstream processing steps [164].

3.2.2 Factors affecting solid-state fermentation of food waste

SSF is a promising method for valorizing FW into valuable bioproducts [167]–[169]. To harness the full potential of SSF for FW valorization, it is essential to understand and optimize the various factors that influence its efficiency. As summarized in Table 5,

key parameters influencing SSF efficiency include pretreatment methods, fungal species, inoculum dosage, pH, temperature, and duration. SSF utilizes fungi like *Aspergillus* and *Trichoderma* to produce hydrolytic enzymes that break down complex FW components into simpler compounds like sugars, amino acids, and fatty acids [169]–[171]. These can be further processed into bioproducts, including bioethanol [172], [173].

Table 5: Parameters and methods for SSF of food waste.

Source	Pretreatment	Enzyme Type	Enzyme Dosage	pH	Temperature (°C)	Hydrolysis Time (h)	Subsequent Process	Ref.
FW	Autoclave	*Starmerella bombicola*	108 (CFU per gram of dry matter)	5.5–7.51	ND	5-6	Anaerobic digestion	[167]
FW	Autoclave	*Pleurotus ostreatus*	10% (w/w)	6.73–8.24	25	ND	Anaerobic digestion	[168]
FW	Drying	*Trichoderma reesei*	106 spores/mL	5	30	5	ND	[169]
FW	Autoclave, Drying	*Serratia marcescens*	ND	NA	ND	2	Prodigiosin production	[170]
FW	Size reduction	*Aspergillus tubingensis*	2×106 spores/mL	4.2	30	5	Biohydrogen production	[171]
FW	Drying	*Bacillus licheniformis*	ND	NA	ND	90h	ND	[172]
FW	Drying	*Aspergillus niger*	2×107 spores per gram of dry material	NA	30	8h	Bioactive compound production	[173]
FW	Size reduction	*Aspergillus oryzae*	106 spores/mL	NA	28	12	Prebiotic production	[174]
FW	Drying	*Aspergillus niger*	106 spores/mL	NA	25	7	ND	[175]
FW	Drying	*Aspergillus awamori*	106 spores/mL	NA	30	65h	Fermentation	[176]
FW	Drying, Autoclave	*Aspergillus niger*	1.0–4.0 × 10^7/g of solid	NA	30	168h	Bioactive compound production	[177]

ND – Not determined, NA – Not adjusted

As shown in Table 5, pretreatment methods like autoclaving or drying are commonly used to prepare the FW substrate for SSF [17], [167], [168], [171]. The choice of fungal species is critical, as different fungi possess varying capabilities to produce hydrolytic enzymes and tolerate different environmental conditions. *Aspergillus* species, particularly *A. niger* and *A. oryzae*, are commonly used in SSF of FW due to their prolific enzyme production and ability to degrade complex carbohydrates [173], [175]. Other fungal species, such as *Trichoderma reesei* and *Pleurotus ostreatus*, have also been explored for their potential in SSF of FW [167], [169].

Inoculum dosage, typically ranging from 106 to 108 spores/mL or g of dry matter, can influence the rate of fungal growth and enzyme production. The pH conditions employed in SSF typically range from 4.2 to 8.24, generally falling within the neutral range, while temperature is typically maintained between 25 and 30 °C, indicating ambient conditions. SSF is often conducted without the need for pH or temperature adjustments, simplifying the process and reducing energy requirements. Fermentation duration varies widely, ranging from hours to several days [170], [173], [174], [177].

3.3 *Integrated solid-state fermentation and enzymatic hydrolysis*

While SSF offers numerous advantages for FW valorization, it also faces challenges, particularly in substrate accessibility and efficient microbial growth and enzyme production, especially in large-scale operations [152]. To overcome these limitations and further enhance the bioconversion process, researchers have explored the integration of SSF with EH. This combined approach leverages the synergistic action of enzymes and microorganisms to maximize the breakdown of complex substrates within FW, leading to the enhanced recovery of valuable compounds such as fermentable sugars and bioactive compounds [178], [179].

3.3.1 Principles of integrated solid-state fermentation and enzymatic hydrolysis

The principle behind integrating SSF and EH lies in maximizing the breakdown of complex substrates within FW, thereby enhancing the recovery of valuable compounds [180], [181]. SSF employs microorganisms to initiate the degradation process, primarily targeting readily accessible carbohydrates, and releasing enzymes that further break down complex molecules into simpler ones [174], [176], [182]. The subsequent EH step utilizes these released enzymes to further depolymerize the remaining recalcitrant complex structures, including cellulose and hemicellulose, into readily fermentable sugars [173], [175]. This synergistic approach leverages the metabolic capabilities of microorganisms and the specificity of enzymes to efficiently valorize FW.

3.3.2 Microorganisms suitable for both solid-state fermentation and enzymatic hydrolysis of food waste

Several microorganisms have been explored for their ability to hydrolyze and ferment FW in SSF. Filamentous fungi, particularly *Aspergillus* species, are frequently employed due to their robust growth on solid substrates and their capacity to secrete a diverse array of hydrolytic enzymes [173]. *A. niger*, in particular, has garnered significant attention due to its prolific enzyme production, including cellulases, xylanases, and pectinases, which are crucial for deconstructing the complex carbohydrates and cell wall structures present in FW. The adaptability of *A. niger* to various fermentation conditions further contributes to its suitability for SSF processes [175], [183].

3.3.3 Products of integrated solid-state fermentation and enzymatic hydrolysis of food waste

The integration of SSF and EH of FW can yield a spectrum of valuable products. The process primarily generates soluble and fermentable carbohydrates, predominantly glucose-rich hydrolysate, about 98% w/w starch to glucose yield, which serve as a carbon source for subsequent LFs to produce biofuels like bioethanol or other value-added chemicals [176]. Concurrently, the SSF process can lead to the release or synthesis of bioactive compounds, including phenolic compounds and antioxidants, which have applications in the food, pharmaceutical, and cosmetic industries [177]. Additionally, the residual solid fraction enriched in lignin can be further utilized as a source of biomaterials or for energy production [174].

3.3.4 Optimization of integrated process parameters

The compatibility of FW with integrated simultaneous SSF and EH was successfully demonstrated without the need for pH or temperature modifications. This aligns with the inherent conditions of both the FW and the microbial cultures used in the process [171], [173]. However, parameters such as inoculum dosage and fermentation time still require optimization to achieve optimal yields [171], [173], [175]. The ability to bypass pH and temperature adjustments represents a significant simplification of the integrated process, potentially facilitating its scalability and industrial adoption [173].

3.4 *Pretreatment procedures of food waste for optimal integrated solid-state fermentation and enzymatic hydrolysis*

Despite the compatibility of FW with integrated SSF and EH in terms of pH and temperature, pretreatment is still necessary to optimize the process and enhance its efficiency [171], [173]. The high moisture content inherent to FW necessitates pretreatment steps to ensure the success of the integrated approach. Excess moisture can hinder microbial growth and enzyme activity during SSF, making drying a crucial initial step. Methods like convective oven drying or hot air drying effectively reduce moisture content to levels suitable for SSF [173], [177].

The size reduction of FW through grinding or milling is another important pretreatment step that enhances the efficiency of SSF and EH. By decreasing particle size, the surface area available for microbial attachment and enzymatic action increases, facilitating the breakdown of complex substrates and improving mass transfer [172], [178].

Additionally, autoclaving the FW prior to fermentation is essential to eliminate competing microorganisms that could interfere with the selected microbial culture's growth and activity. This sterilization step helps maintain a controlled environment, promoting the dominance of the desired microorganism and ensuring efficient enzyme production during SSF. Other pretreatment methods, such as steam explosion, acid/alkali treatment, and microwave irradiation, have also been investigated for

enhancing the efficiency of FW hydrolysis and SSF [177], [174].

4 Gasification and Gas Fermentation

In the pursuit of a circular bioeconomy, where waste streams are transformed into valuable resources, gasification and GF emerge as promising technologies for valorizing the solid residues and wastewater generated from FW treatment processes [178]. Gasification, a thermochemical process, converts carbonaceous feedstocks into a combustible gas mixture known as syngas, primarily composed of hydrogen (H_2) and carbon monoxide (CO) [184]. SCWG, a variation of gasification operating above the critical point of water, offers advantages like higher conversion efficiencies and the ability to handle high-moisture feedstocks, making it particularly suitable for processing fermentation residues and wastewater [185]. GF further utilizes the syngas produced through gasification [184]. By employing acetogenic bacteria, GF can convert syngas into valuable products, including bioethanol [186]. This biotechnological approach provides a crucial link in the TPF concept, enabling the complete valorization of FW and its byproducts into renewable energy.

4.1 *Supercritical water gasification*

SCWG offers a promising avenue for sustainable energy production and environmental remediation by valorizing lignin-rich biomass and FWW [187]. These waste streams are abundant and characterized by high COD and total organic carbon (TOC) content, making them attractive feedstocks for SCWG [188]. The primary goal of SCWG is to convert these waste streams into valuable syngas, a mixture rich in H_2 and CO [185]. The efficacy of SCWG is influenced by several key parameters, including temperature, pressure, residence time, and the choice of catalyst. Catalyst selection is particularly crucial, as it can steer the syngas composition towards either H_2 or CO production [187], [188].

4.1.1 Supercritical water gasification of lignin-rich substrates

Lignin, a complex polymeric component of biomass, presents a challenge for conventional bioconversion processes due to its recalcitrant nature [189]. SCWG, as summarized in Table 6, offers a promising solution for valorizing lignin-rich substrates, such as black liquor, by depolymerizing lignin and converting it into valuable syngas, primarily rich in H_2.

Table 6: Parameters for SCWG of lignin-rich substrates.

Substrate	C/H	Temperature (°C)	Pressure (MPa)	Residence Time (min)	Catalyst	Product	Ref.
Sludge-Lignin	49.7/6.2	650	30	2.9–6.2	ND	CO_2	[190]
Lignin	46.13/5.76	450	24-27	5–40	Ni	H2	[191]
Plastic-Lignin	27.9/3.2	500-750	23-26	5–60	ND	H_2, CO_2	[192]
Lignin	46.13/5.76	450	24-27	5–40	Ni	H2	[193]
Alkali-Lignin	ND	550-850	ND	5	ND	H_2, CO_2	[194]

ND – Not determined

The composition of lignin, particularly its C/H ratio, significantly influences the gasification process and the resulting product distribution. As shown in Table 6, lignin typically exhibits a C/H ratio ranging from 27.9/3.2 to 49.7/6.2 [190], [192]. SCWG of lignin-rich substrates is commonly conducted at temperatures between 450 and 850 °C, under pressures of 23 to 30 MPa, and with residence times spanning from 2.9 to 60 minutes. The utilization of catalysts, particularly nickel-based catalysts, can further enhance the H_2 yield [190]–[194].

4.1.2 Supercritical water gasification of food wastewater

In addition to lignin-rich substrates, FWW, often characterized by high levels of COD and TOC, presents another promising feedstock for SCWG. As summarized in Table 7, these wastewaters typically exhibit COD levels ranging from 38.6 to 217.4 g/L and TOC levels between 37.5 and 60.2 g/L, indicating their substantial organic matter content suitable for conversion [198]. SCWG of FWW has demonstrated the potential to generate valuable syngas rich in H_2 and CO.

Table 7: Parameters for SCWG of food wastewater.

Source	COD	TOC	Temperature (°C)	Pressure (MPa)	Residence Time (min)	Catalyst	Primary Product	Ref.
FWW	5.28×10^4 mg/L	2.17×10^4 mg/L	360–480	28	10–20	Ni-Cu	H_2	[195]
FWW	7300 mg/L	477.3 mg/L	287–683	25	ND	ND	H_2	[196]
FWW	11,650 mg/L	45,813 mg/L	500–700	23–27	1–30	ND	H_2	[197]
FWW	38.6–217.4 g/L	37.5–60.2 g/L	430	235 bar	20s	AlOOH, CeO_2 and Fe_2O_3	CH_4	[198]
FWW	5.48×10^4 mg/L	1.14×104 mg/L	360–480	28	15–45	NaOH, Na_2CO_3, KOH, $K2CO_3$	H_2	[199]
FWW	6.42×10^4 mg/L	1.074×10^4 mg/L	300–500	10–28	20–70	Activated carbon	H_2	[200]
FWW	129,200 mg/L	ND	450–650	23–27	5–30	K_2CO_3, ZnO, and Co_2O_3	H_2	[201]
FWW	6.5–18.6 g/L	2.65–7.85 g/L	600–700	23	24.5–29.1s	NaOH	H_2, CO_2	[202]
FWW	126,490 mg/L	ND	500–700	25	5–40	Na_2CO_3, K_2CO_3	H_2, CO, CH_4	[203]
FWW	25,000 mg/L	10,000 mg/L	400–450	23–25	45	-	H_2	[204]
FWW	ND	ND	420	27.3	60	K_2CO_3	H_2, CO_2, CH_4	[205]
FWW	36950 mg/L	9357 mg/L	500–650	22.5–26.0	0–30	KOH, K_2CO_3, MnO_2, $KMnO_4$	H_2, CO, CH_4, and CO_2	[206]

ND – Not determined

The operating conditions for SCWG of FWW, as shown in Table 7, span a broad range, reflecting the variability in feedstock composition and desired product outcomes. Temperatures typically range from 287 to 700 °C, pressures between 10 and 28 MPa, and residence times from 20 seconds to 70 minutes [195]–[206].

Various catalysts have been explored to promote H_2 production during SCWG of FWW. These include Ni-Cu, activated carbon, and alkali salts such as NaOH, Na_2CO_3, KOH, and K_2CO_3 [18], [200], [206]. Additionally, integrating hydrothermal carbonization (HTC) as a pretreatment step has been shown to significantly enhance gasification efficiency by improving the dewaterability and energy density of the feedstock [204].

4.2 *Gas fermentation of H_2-rich syngas*

The syngas generated from SCWG, particularly the H_2-rich syngas derived from lignin-rich substrates and certain types of FWW, present a valuable feedstock for further bioconversion [189], [207]. GF offers a promising pathway to harness this potential, utilizing microorganisms to convert syngas into valuable biofuels and biochemicals. This biological process leverages the metabolic capabilities of acetogenic bacteria to transform the carbon monoxide, carbon dioxide, and hydrogen present in syngas into a range of products, including ethanol, butanol, and acetic acid [208].

This approach not only contributes to a circular economy by utilizing waste streams but also offers a sustainable alternative to fossil fuels, thus reducing greenhouse gas emissions [209]. The inherent flexibility of syngas fermentation, accommodating diverse feedstocks and gas compositions, including the H_2-rich syngas from SCWG, further accentuates its potential in the pursuit of sustainable energy solutions [189], [207], [209].

4.2.1 Principles of gas fermentation

The conversion of syngas into bioethanol is primarily driven by the metabolic capabilities of acetogenic bacteria [186]. These microorganisms employ the Wood-Ljungdahl pathway (WLP), as illustrated in Figure 6, an ancient and energy-efficient metabolic route, to harness the carbon and energy present in syngas [210]–[212]. This process offers a sustainable alternative by utilizing waste gases or syngas derived from biomass or waste gasification, thereby contributing to a circular economy and reducing greenhouse gas emissions [211].

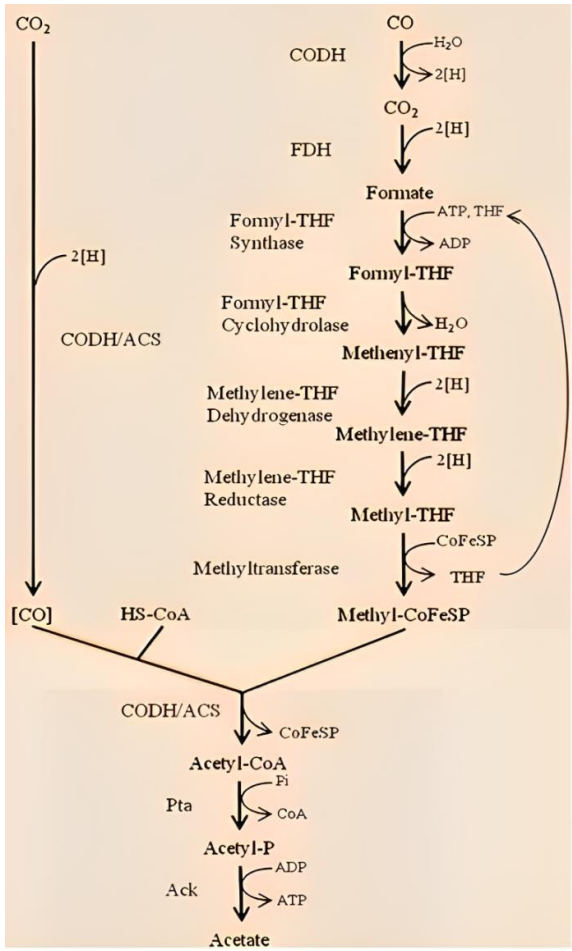

Figure 6: Wood-Ljungdahl pathway (WLP) adapted from Tokuda [212].

The WLP is a complex series of biochemical reactions that enables acetogens to fix carbon from CO or CO_2 and convert it into acetyl-CoA, a central metabolic intermediate [186]. The pathway initiates with the reduction of CO or CO_2 to formate. Formate is then integrated into a tetrahydrofolate-bound C1 unit, which undergoes stepwise reduction and condensation reactions [213]. These reactions culminate in the formation of acetyl-CoA, a crucial building block for various metabolites, including acetate and ethanol. The metabolic fate of acetyl-CoA is intricately regulated by the cell's energy requirements and environmental cues, allowing acetogens to adapt and thrive under varying conditions [186], [208], [211].

The operational parameters employed in GF, as summarized in Table 8, span a range of values, reflecting the diverse metabolic capabilities of acetogens and the adaptability of the bioprocess. In the production of bioethanol, acetyl-CoA can follow two primary pathways. It can be directly reduced to acetaldehyde, followed by a final reduction to ethanol, mediated by a series of enzymes, including aldehyde:ferredoxin oxidoreductase and alcohol dehydrogenase [214]. Alternatively, acetyl-CoA can be converted to acetate, which can then be further utilized as a substrate for LF to produce bioethanol using specialized microorganisms. The inherent flexibility of syngas fermentation, accommodating diverse feedstocks and gas compositions, and the potential for further bioethanol production from acetate, further highlight its potential in the pursuit of sustainable energy solutions [208].

Table 8: Parameters for gas fermentation of CO and H_2-rich syngas.

Syngas Composition	Acetogen	Acetogen Concentration	pH	Temperature (°C)	Pressure	Hydraulic Retention Time (d)	Agitation (rpm)	Primary Products	Ref.
H_2, CO, CO_2, Na	*Clostridium ljungdahlii*	1.23 × 108 cells/mL	5.7	37	2 atm	5.1	300	Acetate	[215]
H_2, CO, CO_2	*Clostridium ljungdahlii, Moorella thermoacetica*	ND	ND	37–60	0.2 MPa	ND	ND	Acetate	[216]
CO, H_2, CO2, N_2	*Clostridium aceticum*	0.5/mL OD600	7.5	30	ND	230h	250	Acetic acid	[217]
CO, H_2, CO_2, N_2	*Clostridium ljungdahlii*	0.61–0.62 g/L CDW	5.9	37	ND	100h	800	Ethanol, acetate	[218]
CO, H_2, CO_2, N_2	*Clostridium autoethanogenum*	0.6–1.67 OD600	6.6–6.7	37	ND	14	200	Ethanol, acetic acid	[219]
CO, H_2, CO_2, N_2	*Clostridium acetobutylicum*	ND	-	37	240 kPa	14	200	SCFA, alcohol	[220]

Table 8: *(Continued)*.

Syngas Composition	Acetogen	Acetogen Concentration	pH	Temperature (°C)	Pressure	Hydraulic Retention Time (d)	Agitation (rpm)	Primary Products	Ref.
CO, H_2, CO_2, CH_4, N_2	*Clostridium butyricum*	ND	4–6	37	ND	16	200	bioethanol	[220]
CO, H_2, CO_2, N_2	*Clostridium aceticum*	0.5 gDM/L	5.6	35	ND	230h	250	Alcohol	[208]
CO, H_2, CO_2, N_2	*Mixed culture*	OD600	4.5–7.5	30–37	1 atm	3	ND	ethanol, acetate, butyrate, caproate	[221]
CO, H_2, CO_2, N_2	*Clostridium aceticum, Clostridium kluyveri*	OD600	6.6–7.5	30	ND	ND	250	n-butyrate, n-caproate, n-butanol	[222]
CO, H_2, CO_2, N_2	*Clostridium carboxidivorans*	OD600	5 - 6	30–37	ND	8	150	ethanol, acetate, butanol, butyrate, hexanol, caproate	[223]
H_2, CO_2	*mixed culture*	ND	7	30	ND	12	120	acetate, formate	[224]
H_2, CO, CO_2	*mixed culture*	ND	4.5–5.5	20–28	ND	ND	ND	acetate	[225]
CO, H_2, CO_2, N_2	*mixed culture*	ND	6.4–6.7	37	1 bar	27	1000	acetic acid	[226]
CO, H2, CO2, CH4	*Saccharomyces cerevisiae*	ND	4–6.5	37	ND	16	200	bioethanol	[227]
CO, H_2, CO_2	*Morella thermoacetica*	OD600	6	60	ND	30	200	acetic acid	[228]
CO, H_2, CO_2, N_2	*Eubacterium callanderi*	0.5/mL OD600	7	37	ND	ND	ND	acetate	[229]

ND – Not determined

4.2.2 Microorganisms for H_2-rich syngas fermentation

Clostridium ljungdahlii stands out as a key player in GF, particularly for its growth on syngas and its natural ability to produce ethanol as a primary metabolite [215], [218]. Its metabolic versatility extends to producing other valuable products like acetate, butanol, and even longer-chain alcohols under specific conditions [223]. Additionally, its genetic tractability allows for metabolic engineering to enhance ethanol production and expand its product range. Other acetogens like *Clostridium aceticum* and *Clostridium carboxidivorans* have also been explored, showcasing the diverse microbial potential for GF [217], [223].

4.2.3 Products of H_2-rich syngas fermentation

Syngas fermentation exhibits remarkable versatility in product formation, extending beyond bioethanol to encompass a range of short-chain fatty acids (SCFAs) and bioalcohols [209]. Acetic acid, butyric acid, and hexanoic acid are prominent SCFAs produced during the acidogenic phase, while ethanol, butanol, and hexanol are characteristic products of the solventogenic phase [208]. The specific product profile is influenced by factors such as bacterial strain, gas composition, and operating conditions. Particularly, *C. ljungdahlii* exhibits a propensity for ethanol production, whereas *C. carboxidivorans* is recognized for its ability to generate a broader spectrum of alcohols, including butanol and hexanol [208], [222].

4.2.4 Optimization of H_2-rich syngas fermentation parameters

The operational parameters employed in syngas fermentation, as summarized in Table 8, span a range of values, reflecting the diverse metabolic capabilities of acetogens and the adaptability of the bioprocess. Acetogen concentrations, often measured in optical density (OD) or cell dry weight (CDW), typically range from 0.2 to 2.6 OD600 nm or 0.34 to 0.62 g/L

CDW [208], [219]. The pH, a critical factor influencing metabolic pathways, is generally maintained between 4.5 and 7.5, with lower pH favoring solventogenesis and higher pH promoting acidogenesis [215], [222]. The temperature range for syngas fermentation is typically between 30 and 60 °C, with 37 °C being commonly used for mesophilic acetogens [217], [219]. Pressure conditions can vary from atmospheric to elevated levels, with some studies reporting pressures up to 2 atm [215]. Hydraulic retention times (HRTs) in continuous cultures typically range from 5 to 29 days, while agitation speeds in bioreactors are usually maintained between 120 and 1000 rpm [208], [209].

A closer examination of Table 8 reveals interesting comparisons. For instance, focusing on *Clostridium ljungdahlii*, under similar temperature conditions of 37 °C, a pH of 5.7 and a pressure of 2 atm resulted in acetate as the primary product [216], whereas a slightly higher pH of 5.9 and no added pressure led to a mixture of ethanol and acetate [218]. This suggests that pH and pressure can significantly influence product selectivity. Comparing different bacteria under similar conditions, *Clostridium aceticum* at 30 °C and pH 7.5 produced acetic acid [216], while a mixed culture at 30–37 °C and pH 4.5–7.5 produced a wider range of products, including ethanol, acetate, butyrate, and caproate [221]. This highlights the impact of microbial species on product profiles. Furthermore, the long hydraulic retention time of 230 hours for *C. aceticum* [218] compared to 5.1 days (122.4 h) for *C. ljungdahlii* [215] might explain the higher concentration of products observed in the case of *C. ljungdahlii*, indicating the potential importance of residence time in product accumulation. Lastly, the use of *S. cerevisiae* in syngas fermentation demonstrates the potential for metabolic diversity [227]–[229].

5 Submerged (liquid) Fermentation

The glucose-rich hydrolysate obtained from the integrated SSF and EH of FW, as discussed in the previous section, serves as an ideal substrate for further bioethanol production through liquid-phase fermentation. This process, also known as liquid fermentation, involves the conversion of readily available sugars into ethanol under anaerobic or microaerobic conditions, primarily facilitated by yeast [230]. The high sugar content in the hydrolysate, resulting from the efficient breakdown of complex carbohydrates during the previous steps, presents a significant advantage for LF, potentially leading to higher ethanol yields and productivity compared to fermentations using raw or minimally pretreated feedstocks [231]. However, optimal yeast growth and sugar utilization are influenced by several factors, including nutrient availability, pH and temperature, and the presence of potential inhibitors from pretreatment. Maintaining these factors within optimal ranges is crucial for maximizing ethanol production [232]–[234].

5.1 *Principles of submerged fermentation*

LF is a widely employed bioprocess that utilizes microorganisms, primarily yeast, to convert sugars into ethanol. This process involves a series of biochemical reactions that occur within a liquid medium, where the microorganisms are suspended [232]. The initial step involves the breakdown of complex carbohydrates, such as starch or cellulose, into simpler sugars like glucose, typically through EH or other pretreatment methods. These readily available sugars are then metabolized by the yeast under anaerobic or microaerobic conditions [152].

The metabolic pathway responsible for ethanol production in yeast is primarily glycolysis, followed by alcoholic fermentation. During glycolysis, glucose is converted into pyruvate, generating a small amount of ATP (cellular energy) and NADH (reducing power). In the absence of oxygen, pyruvate is further converted to acetaldehyde, releasing carbon dioxide. Finally, acetaldehyde is reduced to ethanol by alcohol dehydrogenase, regenerating NAD+ for continued glycolysis [232], [233].

This LF process offers several advantages, including its relative simplicity, scalability for industrial applications, cost-effectiveness due to the use of readily available and robust microorganisms like yeast, and flexibility in utilizing diverse feedstocks, including first and second-generation biomass [97], [232]. This flexibility makes LF particularly attractive for valorizing various waste streams, including those derived from FW [14]. However, the efficiency of LF is highly dependent on the accessibility of sugars in the feedstock. Therefore, proper pretreatment methods, such as EH and SSF, are often necessary to break down complex carbohydrates and release fermentable sugars, ensuring optimal ethanol yields [234].

5.2 *Submerged fermentation of glucose-rich hydrolysate*

The glucose-rich hydrolysate obtained from the integrated SSF and EH of FW serves as an ideal substrate for further bioethanol production through LF. This process leverages the metabolic capabilities of microorganisms, primarily yeast, to efficiently convert the readily available simple sugars into ethanol under anaerobic conditions. The high sugar content in the hydrolysate, resulting from the efficient breakdown of complex carbohydrates during the previous steps, presents a significant advantage for LF, potentially leading to higher ethanol yields and productivity compared to fermentations using raw or minimally pretreated feedstocks [97], [232], [233]. The diverse range of microorganisms and process conditions employed in LF of glucose-rich hydrolysates for bioethanol production are summarized in Table 9.

Table 9: Parameters for submerged fermentation of sugar-rich substrates.

Sugar Type	Yeast Type	Yeast Concentration	pH	Temperature (°C)	Fermentation Time (d)	Agitation (rpm)	Ref.
Glucose, xylose	*Saccharomyces cerevisiae*	5% v/v	6.5	40	6	150	[235]
Glucose	*Escherichia coli (bacteria)*	-	-	37	29 h	200	[236]
Glucose, xylose	*Saccharomyces cerevisiae, Scheffersomyces stipitis*	0.1% (w/v)	5	30	2, 5	200	[237]
Glucose	*Saccharomyces cerevisiae*	2% (w/v)	-	37	4	-	[238]
Glucose	*Saccharomyces cerevisiae*	10% (v/v)	5	30	30 h	100	[239]
Glucose	*Saccharomyces cerevisiae*	1% (v/v)		30	12 h	150	[240]
Glucose	*Saccharomyces cerevisiae*	7.25 × 106 cells/mL	4.8	35	1	120	[241]
Glucose	*Kluyveromyces marxianus, Pichia kudriavzevii*	-	4.8	30	3	150	[26]
Reducing sugar	*Saccharomyces cerevisiae*	dry yeast: water: glucose (100:1000:1, w/w)		30	50 h	400	[242]
Glucose	*Saccharomyces cerevisiae*	1 g/L	5	32	2	130	[243]
Glucose	*Saccharomyces cerevisiae*	2% (v/v)	6	30	1	200	[244]
Glucose	*Saccharomyces cerevisiae*	10% (w/v)	5	30	4	-	[245]
Reducing sugar	*Saccharomyces cerevisiae*	4.0 × 107 cells/mL	-	28	7	-	[246]
Reducing sugar	*Saccharomyces cerevisiae*	-	-	45	2	-	[247]
Glucose	*Saccharomyces cerevisiae*	2.5 x 105 CFU/mL	4-5	28	32 h	-	[248]
Reducing sugar	*Saccharomyces cerevisiae, Pichia Stipitis*	24 × 106 cells/ml, 26 × 106 cells/ml	5.5	35	5	150	[130]
Reducing sugar	*Saccharomyces cerevisiae, Pichia Stipitis*	10% (v/v)	4.5	30	3	-	[249]
Glucose	*Saccharomyces cerevisiae*	5% (v/v)	3	30	1	150	[250]

5.2.1 Microorganisms for submerged fermentation of glucose-rich hydrolysate

The success of LF for bioethanol production relies heavily on the selection of appropriate microorganisms. As shown in Table 9, *Saccharomyces cerevisiae* is the predominant microorganism utilized in the studies reviewed for the fermentation of glucose-rich substrates to bioethanol. This yeast's high ethanol tolerance and efficient glucose metabolism make it well-suited for industrial bioethanol production [245]. While other microorganisms, such as *Escherichia coli*, *Scheffersomyces stipitis, Kluyveromyces marxianus*, and *Pichia kudriavzevii* have been explored, *S. cerevisiae* remains the preferred choice due to its established track record and favorable fermentation characteristics.

5.2.2 Optimization of submerged fermentation parameters of glucose-rich hydrolysate

The fermentation process parameters, including yeast concentration, pH, temperature, fermentation time, and agitation, play a crucial role in determining ethanol yield and productivity, as summarized in Table 9. Yeast concentrations typically range from 0.1% to 10% (w/v or v/v), with some studies using a low concentration of 0.1% (w/v) [237] while others employ a higher concentration of 10% (v/v) [238], the

optimal concentration depending on the specific strain and substrate conditions. The pH is generally maintained between 3 and 6.5, providing a suitable environment for yeast growth and ethanol production. Temperature control is also crucial, with most studies operating within the mesophilic range of 30–40 °C. Fermentation time can vary significantly, from as short as 12 hours to several days, depending on the substrate concentration, yeast strain, and desired ethanol yield. Agitation, typically between 100 and 400 rpm, ensures adequate mixing and mass transfer within the fermentation broth [235]–[250].

5.3 *Submerged fermentation of acetate-rich broth*

In cases where GF employs microorganisms like *Clostridium aceticum*, the primary product is often acetate rather than ethanol [251], as detailed in Table 10. To further valorize this acetate-rich broth and maximize bioethanol yield, LF can be employed as a subsequent step. This process utilizes specialized microorganisms capable of converting acetate into ethanol, thus offering a sustainable and economically viable route for biofuel generation [97], [252]. The abundance of acetate as a byproduct in various industrial processes, including biodiesel production and lignocellulosic biomass pretreatment, further strengthens its appeal as a low-cost feedstock for microbial fermentation [253]. The conversion of acetate to ethanol not only addresses the challenge of waste valorization but also contributes to a circular bioeconomy by reducing reliance on traditional sugar-based feedstocks [254].

Table 10: Parameters for submerged fermentation of sugar-rich substrates.

Yeast type	Yeast Concentration	pH	Temperature (°C)	Fermentation Time (d)	Agitation (rpm)	Ref.
Saccharomyces cerevisiae	OD660	5	30	-	-	[255]
Escherichia coli	-	-	37	200h	150	[256]
Clostridium tyrobutyricum (acetogen)	-	-	35	12–45.8h (HRT)	-	[257]
Kluyveromyces marxianus	OD620	-	30	2	180	[258]
Kluyveromyces marxianus	OD620	-	30–40	-	180	[253]

5.2.2 Microorganisms for submerged fermentation of acetate-rich broth

Saccharomyces cerevisiae, a workhorse in industrial biotechnology, has garnered significant attention for its potential in acetate-based bioethanol production. Its inherent ability to metabolize glucose efficiently makes it an appealing candidate for simultaneous glucose-acetate fermentation, enhancing overall carbon conversion efficiency and potentially improving ethanol yields. Moreover, the introduction of heterologous pathways, such as the acetylating acetaldehyde dehydrogenase (A-ALD) pathway, has further empowered *S. cerevisiae* to reduce acetate to ethanol under anaerobic conditions, showcasing its metabolic flexibility and adaptability for acetate-rich fermentations [255].

5.2.2 Optimization of submerged fermentation parameters of acetate-rich broth

Yeast concentrations typically range from 1 to 3 OD600, ensuring sufficient biocatalytic activity while minimizing substrate loss to biomass formation [255], [253]. The pH is often maintained between 5 and 5.5, providing a favorable environment for yeast growth and metabolism while mitigating the inhibitory effects of acetate [255], [256]. Temperature control between 30 and 40 °C further supports optimal yeast performance, with some studies reporting enhanced acetate utilization at elevated temperatures [252]. Fermentation times vary depending on substrate concentration, strain characteristics, and desired product yields, typically ranging from 1 to 7 days. Agitation speeds of 150 to 200 rpm ensure adequate mixing and mass transfer, promoting efficient substrate utilization and product formation.

6 Simultaneous Co-Fermentation

Co-fermentation is a bioprocessing strategy that employs multiple microbial strains to synergistically convert a complex substrate into a desired product. In the context of bioethanol production, this approach often involves the use of *Aspergillus* species for starch hydrolysis, *Saccharomyces cerevisiae* for hexose fermentation, and *Clostridium* species for pentose fermentation [254]. The combined metabolic capabilities of these microorganisms enable the efficient utilization of diverse sugars present in

lignocellulosic biomass, leading to improved ethanol yields.

6.1 *Simultaneous co-fermentation of solid-liquid phase*

The simultaneous co-fermentation of FW hydrolysate using *Aspergillus awamori* and *Saccharomyces cerevisiae* in a solid-liquid system has been investigated for bioethanol production. This approach leverages the amylolytic activity of *A. awamori* to hydrolyze starch into fermentable sugars, which are then directly converted to ethanol by *S. cerevisiae* in the same bioreactor. A maximum ethanol concentration of 1.13% (v/v) was reported using this simultaneous saccharification and fermentation SSF technique. Further optimization, by adding additional *S. cerevisiae* after 8 hours of fermentation, led to a significant increase in ethanol concentration to 3.985% (v/v), demonstrating the potential for improving yields through process modifications [248].

6.2 *Simultaneous co-fermentation of liquid-gas phase*

Another co-fermentation strategy involves the use of *Saccharomyces cerevisiae* and *Clostridium beijerinckii* in a liquid-gas system for bioethanol production from lignocellulosic hydrolysate. In this approach, *S. cerevisiae* first rapidly ferments glucose to ethanol, creating a favorable anaerobic environment for the subsequent fermentation of pentoses (xylose and arabinose) to ethanol and butanol by *C. beijerinckii*. By optimizing the co-culture conditions, an ethanol production of 20.8 g/L was achieved, showcasing the effectiveness of this strategy in utilizing both hexose and pentose sugars from the hydrolysate [259].

6.3 *Simultaneous tri-phase co-fermentation*

While solid-liquid and liquid-gas co-fermentation systems have shown promise, the potential of a simultaneous tri-phase (solid-liquid-gas) fermentation utilizing *Aspergillus*, *Saccharomyces*, and *Clostridium* for bioethanol production remains largely unexplored. This innovative approach could integrate the advantages of *Aspergillus*-mediated starch hydrolysis in the solid phase, *Saccharomyces*-driven hexose fermentation in the liquid phase, and *Clostridium*-mediated pentose fermentation in the gas phase, potentially leading to even higher ethanol yields and process efficiency. However, realizing the full potential of tri-phase co-fermentation will require overcoming challenges such as maintaining optimal conditions for each microorganism, managing mass transfer limitations in a three-phase system [260], and understanding the complex interactions between the different microbial communities.

7 Integrated Process of Tri-Phase Fermentation

The integration of various bioconversion technologies offers a promising approach to maximize the valorization of FW and its byproducts for sustainable bioethanol production [97]. By combining different processes, such as EH, SSF, gasification, and GF, it is possible to achieve higher yields, improved efficiency, and a more comprehensive utilization of resources. Figure 7 provides a visual representation of these various integrated process configurations, showcasing the potential to create a synergistic and sustainable bioethanol production system. This section explores these integrated process configurations in detail, ranging from step-wise approaches to simultaneous tri-phase co-fermentation, highlighting their potential benefits and challenges.

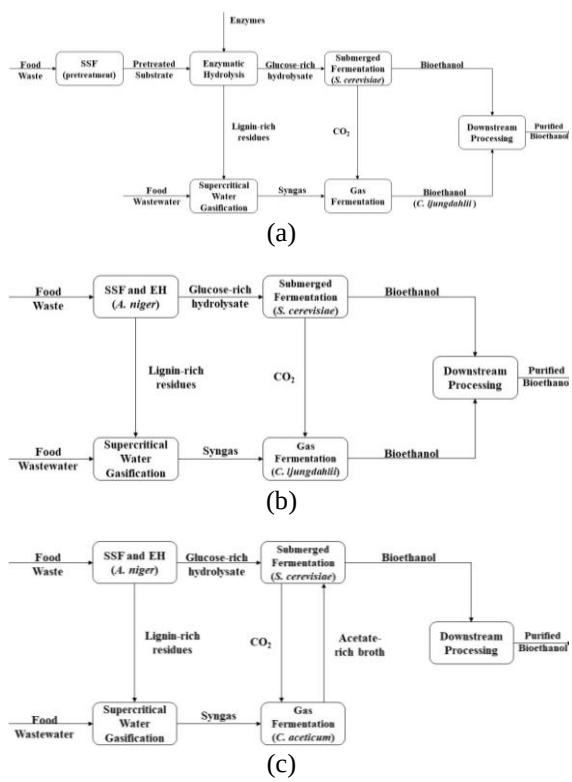

Figure 7: Integrated TPF for bioethanol production from FW.

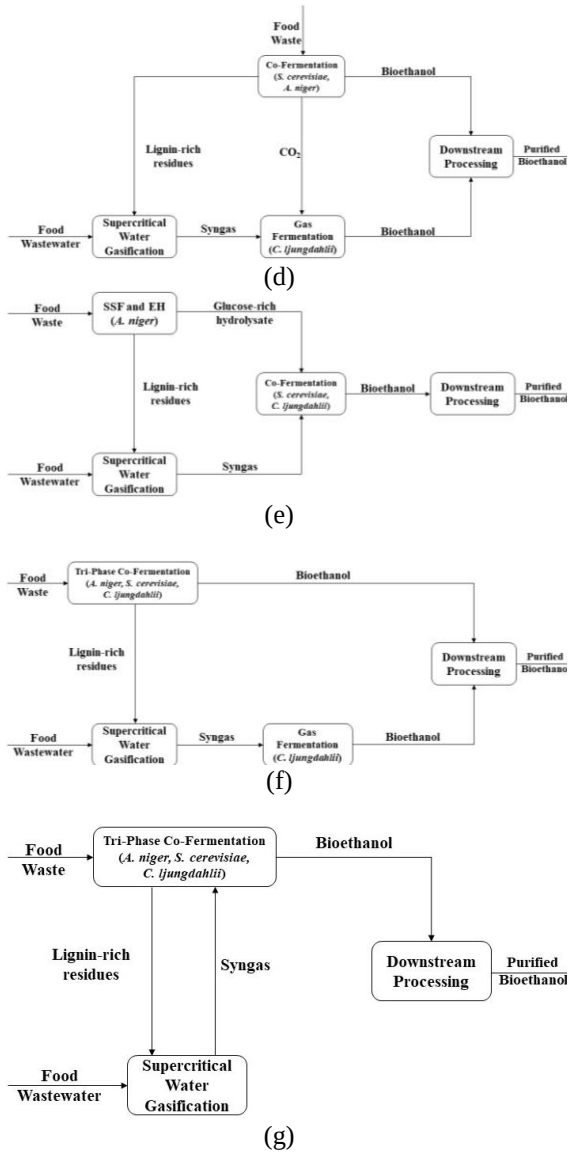

Figure 7: *(Continued)*.

7.1 *Step-wise tri-phase fermentation (Figure 7(a))*

FW is first pretreated and then subjected to SSF using *A. niger* for enzyme production. This is followed by EH, potentially with supplemental enzyme addition. After liquid-solid separation, the liquid fraction undergoes LF with *S. cerevisiae*, followed by downstream processing. The solid residue is subjected to SCWG and GF using *C. ljungdahlii* to produce bioethanol, which is then processed alongside the LF products.

7.2 *Integrated solid-state fermentation and enzymatic hydrolysis (Figure 7(b))*

Streamlining the step-wise process, this approach combines SSF and EH into a single step using *A. niger*, eliminating the need for separate hydrolysis.

7.3 *Simultaneous submerged glucose-acetate fermentation (Figure 7(c))*

Building on the integrated SSF-hydrolysis, this approach introduces the concurrent fermentation of glucose from SSF and acetate from GF. Potential GF products are combined with SSF products before entering LF.

7.4 *Simultaneous solid-liquid co-fermentation (Figure 7(d))*

Further integrating the process, this strategy employs the simultaneous fermentation of *A. niger* and *S. cerevisiae* within a single reactor, where SSF and liquid hydrolysis occur concurrently.

7.5 *Simultaneous liquid-gas co-fermentation (Figure 7(e))*

This approach merges liquid and GF phases into a single process, where *S. cerevisiae* and *C. ljungdahli*i work together to convert sugars and gases into bioethanol. This streamlined configuration could potentially benefit from a bubble column reactor to enhance solid-liquid mass transfer and promote synergistic interactions [261].

7.6 *Non-looped simultaneous tri-phase co-fermentation (Figure 7(f))*

This strategy represents the pinnacle of process integration, combining solid-state, liquid, and GF into a single bioconversion process. All three microorganisms—*Aspergillus*, *Saccharomyces*, and *Clostridium*—coexist and interact within a single reactor, maximizing synergistic effects and resource utilization. The solid residues (lignin) undergo SCWG and GF for further bioethanol production. To optimize this tri-phase co-fermentation, future research could explore the incorporation of a gas-solid reactor to potentially improve gas-solid contact and mass transfer efficiency [30], [261].

7.7 *Looped simultaneous tri-phase co-fermentation (Figure 7(g))*

This process builds on the non-loop tri-phase co-fermentation by introducing a circular element, looping the products of SCWG and GF back into the main co-fermentation reactor. This configuration aims to maximize resource recovery and process efficiency. Looking forward, a combined bubble and gas-solid reactor design could further enhance this looped system [262], creating a truly synergistic and sustainable bioethanol production platform.

8 Techno-Economic Analysis (TEA)

A comprehensive techno-economic analysis (TEA) is essential to evaluate the feasibility and sustainability of TPF for bioethanol production from FW [263]. This section explores the technological and economic challenges associated with this process.

8.1 *Technological challenges of TPF*

The successful implementation of TPF for bioethanol production from FW hinges on overcoming various technological hurdles, as summarized in Table 11. These challenges encompass strain engineering and fermentation optimization to enhance microbial performance and product yields, efficient downstream processing and purification techniques to recover valuable products, effective waste management and byproduct utilization strategies to minimize environmental impact, and robust sensitivity analysis and uncertainty quantification to ensure process reliability. Additionally, scaling up the process from laboratory to industrial levels while maintaining consistency and efficiency poses a significant challenge. This section delves into these technological challenges and highlights potential solutions and research directions to advance the field [174], [235], [264], [265].

Table 11: Parameters for submerged fermentation of sugar-rich substrates.

Technological Challenges	SSF (*Aspergillus niger*)	Liquid (*Saccharomyces cerevisiae*)	Gas (*Clostridium ljungdahlii*)
Strain Engineering and Fermentation	Optimizing strain for increased product yields and tolerance	Enhancing ethanol yields and tolerance through strain engineering	Improving strain for higher ethanol yields and industrial application
Downstream Processing and Purification	Complex matrix necessitates selective extraction and purification techniques	Selective separation of multiple fermentation products	Efficient recovery and purification of bioethanol from mixed products
Waste Management and Byproduct Utilization	Valorization of residual solid waste	Valorization of residues and byproducts	Management and potential valorization of byproducts and residual biomass
Sensitivity Analysis and Uncertainty Quantification	Impact of substrate composition, fermentation conditions on product quality	Optimization of co-fermentation parameters for ethanol production	Impact of gas composition and impurities on fermentation performance
Scale-up and Commercialization	Heat and mass transfer limitations, contamination, and process control	Maintaining productivity, yield, and product quality at large scale	Mass transfer limitations and process control during scale-up
Comparison with Existing Processes	Sustainable alternative for bioactive compound production	Sustainable alternative utilizing waste streams	Sustainable alternative to traditional bioethanol production methods
References	[173]–[177], [264]	[235], [237], [243], [244], [255]	[215], [216], [218]

8.1.1 Strain engineering and fermentation optimization

Strain engineering and fermentation optimization offer significant opportunities to enhance the performance of microorganisms involved in bioethanol production from FW. The wild-type *A. niger* GH1 strain has been shown to increase free phenols in pineapple waste during the first few hours of fermentation [173]. However, further advancements can be achieved through strain engineering techniques, such as overexpressing genes encoding key enzymes or disrupting genes responsible for phenolic compound degradation [173], [177].

S. cerevisiae, a workhorse in industrial ethanol production, can also be further improved through strain engineering which can result in high osmotolerance of the *S. cerevisiae* KL17 strain, achieving high ethanol titers and yields close to the theoretical maximum, even in the presence of fermentation inhibitors [236].

Similarly, while the ability of *C. ljungdahlii* to convert syngas into ethanol and acetate is well-recognized, its industrial application necessitates further advancements. Studies have shown its potential for producing ethanol from both beech wood and lignin-derived syngas, but the low ethanol yields highlight the need for strain improvement through metabolic engineering and fermentation optimization [215], [217].

8.1.2 Downstream processing and purification

Efficient downstream processing and purification methods are essential for recovering and purifying bioethanol and other valuable products from fermentation broths. The presence of multiple fermentation products necessitates selective separation techniques. Conventional methods such as solvent extraction, filtration, and lyophilization have been employed, but these can be time-consuming and involve the use of large volumes of organic solvents. Exploring advanced techniques like membrane filtration, chromatography, and supercritical fluid extraction could improve efficiency and selectivity in product recovery. Pervaporation using a PDMS/PEI hollow-fiber membrane has been explored for the separation of ethanol and xylose, achieving high ethanol recovery rates [237].

8.1.3 Waste management and byproduct utilization

Efficient waste management and byproduct utilization are crucial for the economic and environmental sustainability of bioethanol production from FW. The SSF process generates residual solid waste that, while requiring proper management, can be further utilized as animal feed, a source of dietary fiber, or through composting or anaerobic digestion for biogas production [174]. Similarly, liquid and solid residues from fermentation processes can also be valorized to achieve high biomethane potentials from the anaerobic digestion of these residues. The syngas fermentation process also generates byproducts and residual biomass [243], [244]. While efficient carbon utilization has been reported, further research is needed to explore the potential for utilizing the residual biomass or CO_2 for other value-added applications [218].

8.1.4 Sensitivity analysis, scale-up, and commercialization

Sensitivity analysis and uncertainty quantification are vital for assessing the robustness and reliability of the bioprocesses involved in bioethanol production from FW. The impact of various factors on process performance and product quality includes substrate composition, fermentation conditions, and even the choice of microbial strains. Conducting sensitivity analyses across these diverse bioprocesses aids in identifying critical parameters and developing strategies to mitigate potential risks and uncertainties, which is crucial for successful scale-up and commercialization [176], [218], [235], [264].

Scaling up fermentation processes from lab-scale to industrial levels presents challenges in maintaining consistency, efficiency, and product quality. Factors such as heat and mass transfer limitations, contamination risks, and process control become increasingly important as the scale increases. While successful scale-up has been demonstrated for some bioprocesses, further research and development are necessary to optimize and validate the scalability of TPF for bioethanol production from FW [173]–[177].

8.1.5 Comparison with existing processes

Comparing the SSF process using *A. niger* with existing methods for bioactive compound production highlights its potential as a sustainable and eco-friendly alternative that valorizes FW [173], [175]. Similarly, bioethanol production from glucose and acetate-rich substrates using *S. cerevisiae* demonstrates the sustainability benefits of utilizing waste streams [177], [244]. Additionally, bioethanol production from syngas using *C. ljungdahlii* underscores the potential of syngas fermentation as a sustainable alternative to traditional methods reliant on food crops [218].

Bioethanol production using these diverse microbial systems offers promising sustainable alternatives. Strain engineering and optimization of fermentation conditions hold the potential for enhancing yields and tolerance to challenging industrial conditions [173], [218], [244]. Furthermore, efficient downstream processing and purification techniques, coupled with waste management and byproduct utilization strategies, are crucial for achieving economic viability and environmental sustainability. Sensitivity analysis and uncertainty quantification will also aid in identifying critical parameters and mitigating potential risks. While scale-up

and commercialization remain challenging, successful demonstrations of large-scale microbial oil production provide optimism [176].

8.2 *Economic challenges*

While TPF offers a promising pathway for sustainable bioethanol production from FW, its economic feasibility is crucial for its widespread adoption and commercialization. Table 12 summarizes some of the key economic considerations associated with bioethanol production from FW. This section explores these economic challenges in more detail, focusing on the costs of microorganism production, enzyme utilization, and pretreatment. Furthermore, it emphasizes the importance of conducting comprehensive techno-economic analyses to compare TPF with existing processes and evaluate its overall economic viability and environmental impact [174], [235], [264].

Table 12: Parameters for submerged fermentation of sugar-rich substrates.

Economic Challenges	SSF (*Aspergillus niger*)	Liquid (*Saccharomyces cerevisiae*)	Gas (*Clostridium ljungdahlii*)
Microorganism Production and Costs	Enzyme production costs can be high; exploring in-house production or alternative strategies	High cost of commercial yeast; need for cost-effective alternatives	Eliminates the need for exogenous enzymes, potentially reducing costs
Pretreatment Costs	Pretreatment costs can vary; optimization needed to balance cost and efficiency	Extensive pretreatment is often required, impacting costs	Minimal pretreatment is needed, potentially lowering costs
Fermentation Efficiency and Productivity	Need for further research on yields and productivity for economic assessment	Strain selection and process optimization are key for high yields and productivity	Low ethanol titers currently; optimization of gas composition, pH, and HRT is crucial
Downstream Processing Costs	Downstream processing can be costly; exploring alternative techniques may improve economic feasibility	Need for efficient and cost-effective ethanol recovery and purification	Selective separation of metabolites is necessary; exploring alternatives to distillation for cost reduction
Capital and Operational Costs	Lab-scale focus currently limits economic analysis; scale-up considerations are crucial	Capital and operational costs are significant; byproduct valorization can help offset costs	High capital investment for equipment; operational costs are substantial; process integration offers cost reduction
References	[173]–[177], [264]	[235], [237], [243], [244], [255]	[215], [216], [218]

8.2.1 Microorganism and enzyme costs

The economic feasibility of bioprocesses, such as SSF and fermentation, is significantly influenced by the costs associated with microorganism production and enzyme utilization. While *A. niger* has shown promise in producing valuable enzymes from FW, a thorough economic assessment is needed to evaluate the cost-effectiveness of this approach compared to using commercial enzymes [173], [175]. The high cost of commercial enzymes underscores the importance of developing cost-effective in-house enzyme production methods or exploring alternative strategies, such as utilizing fungal strains for enzyme production from waste streams [244]. Syngas fermentation using *C. ljungdahlii* offers a potential advantage in this regard, as it eliminates the need for exogenous enzymes, thus reducing costs [218].

8.2.2 Pretreatment and feedstock costs

Pretreatment of lignocellulosic biomass, although essential for enhancing substrate accessibility, can significantly impact the overall process economics. While simple pretreatment methods like drying and grinding can potentially reduce costs, their impact on fermentation efficiency and overall economic feasibility requires further evaluation. Similarly, the cost of the feedstock itself, whether FW, lignocellulosic biomass, or syngas, plays a crucial role in determining the economic viability of the process [173], [174].

8.2.3 Fermentation efficiency and productivity

Fermentation efficiency and productivity directly influence the economic viability of bioethanol production. Achieving high ethanol titers, yields, and

productivity is essential to maximize output and minimize costs. Strain selection, process optimization, and the utilization of mixed carbon sources, as demonstrated in some studies, can contribute to improved fermentation performance [255].

8.2.4 Downstream processing costs

Downstream processing and purification represent a significant portion of the overall production costs. Efficient and cost-effective methods for recovering and purifying bioethanol and other value-added products are essential. While conventional methods like distillation and solvent extraction have been employed, exploring alternative separation technologies such as pervaporation or adsorption could potentially reduce energy consumption and improve economic feasibility [237, 177].

8.2.3 Capital and operational costs

The capital investment required for bioreactors, gasifiers, and downstream processing equipment, along with the operational costs associated with feedstock preparation, fermentation, and product recovery, significantly impacts the economic viability of bioethanol production from FW. While process intensification and the utilization of waste streams for energy or nutrient recovery can potentially offset some costs [215]–[218].

8.3 *The tri-phase advantages and disadvantages*

As evident from the techno-economic analysis, each fermentation process presents its own set of technological and economic hurdles. However, these challenges exhibit a complementary nature, suggesting the potential for a synergistic solution. While SSF may face limitations in substrate accessibility and mass transfer, EH can enhance the release of fermentable sugars. Similarly, while GF offers a cost-effective way to utilize syngas, it might be limited by low product titers, which can be addressed through subsequent LF. The integration of these processes in a TPF system could potentially leverage the strengths of each phase while mitigating their weaknesses. This synergistic approach not only maximizes resource utilization and promotes a circular economy but also offers a pathway to overcome the technological and economic barriers hindering the widespread adoption of bioethanol production from FW.

9 Life Cycle Assessment (LCA)

A comprehensive life cycle assessment (LCA) is essential to evaluate the environmental sustainability of bioethanol production from FW using TPF. This approach involves considering the potential impacts at each stage of the process, from feedstock acquisition to final product disposal [244].

9.1 *Feedstock acquisition and pretreatment*

Utilizing FW as a feedstock presents logistical challenges in terms of collection and transportation, potentially increasing the carbon footprint [174]. The pretreatment stage, often involving chemicals or energy-intensive processes, can also generate environmental burdens. While EH offers a milder alternative to acid pretreatment, it still involves the production and transportation of enzymes, adding to the overall environmental impact. Therefore, developing eco-friendly and energy-efficient pretreatment technologies is crucial for minimizing the environmental footprint of this stage [134].

9.2 *Fermentation and downstream processing*

The fermentation stage, where sugars are converted into ethanol, is significantly influenced by the choice of microorganisms and process conditions. The use of genetically modified organisms (GMOs) can raise concerns about potential environmental risks, necessitating careful risk assessment and management strategies. Additionally, energy consumption and emissions from maintaining optimal fermentation conditions in bioreactors need to be considered and minimized. Downstream processing, which involves the separation and purification of bioethanol, also carries environmental implications [237].

9.3 *Waste management and byproduct utilization*

Proper management of byproducts and residues generated during TPF is essential to minimize environmental impact and promote a circular economy. The SSF process generates residual solid waste that can be further valorized through composting, anaerobic digestion, or animal feed, thus reducing waste and contributing to additional energy or nutrient recovery. The gasification and GF stages also produce byproducts and residual biomass that require proper management and potential valorization [174], [242], [244].

10 Future Directions for Tri-Phase Fermentation

The promising potential of TPF for sustainable bioethanol production from FW presents several exciting avenues for future research and development. Central to these efforts is the realization of a true simultaneous tri-phase co-fermentation system. This would involve the harmonious coexistence and interaction of *Aspergillus*, *Saccharomyces*, and *Clostridium* within a single bioreactor, enabling concurrent hydrolysis alongside solid-state, liquid, and gas fermentation. Such a breakthrough could revolutionize resource utilization and significantly enhance bioethanol yields. However, achieving this requires tackling challenges in reactor design, microbial consortia engineering, process control, and nanotechnology integration.

Beyond the core microbial trio, exploring the integration of other commercially available bacterial groups could further enhance TPF's efficiency and product diversity. For example, incorporating strains of *Zymomonas mobilis*, known for their rapid sugar uptake and high ethanol yields, could potentially boost the liquid fermentation phase. Similarly, including cellulolytic bacteria like *Clostridium thermocellum* or *Thermoanaerobacterium saccharolyticum* might enhance the breakdown of complex carbohydrates in the solid phase, reducing the reliance on fungal enzymes. The cost-effectiveness of using these additional bacterial groups would need to be carefully evaluated, considering factors such as their growth requirements, fermentation performance, and potential for genetic manipulation. While some strains might offer superior performance, their higher cost compared to, for instance, readily available *Saccharomyces cerevisiae* strains, could impact the overall economic feasibility of the process. A thorough cost-benefit analysis, integrated within the TEA, would be essential to determine the optimal microbial consortium for TPF.

Innovative reactor designs, such as integrated packed-bed and bubble column reactors or gas-solid contact systems, must be explored to optimize mass transfer and microbial interactions in a tri-phase environment. Advanced configurations, like microfluidic or rotating biofilm reactors, could intensify mass transfer, boosting process efficiency. In parallel, microbial consortia engineering will play a critical role. Using synthetic biology, communication pathways between microbial species could be enhanced, while adaptive laboratory evolution may help select consortia with superior stability and performance. Mathematical modeling would further aid in predicting and optimizing complex microbial interactions. Real-time monitoring and control systems, leveraging biosensors and artificial intelligence, are essential to ensure stable operations in tri-phase bioreactors. Moreover, integrating nanotechnology offers immense promise—nanocatalysts could improve enzymatic hydrolysis and gas fermentation, nanoparticles might enhance mass transfer, and nanomaterials could mitigate inhibitory byproducts or deliver nutrients to optimize microbial activity.

Another direction involves engineering microorganisms capable of performing all three fermentation phases within a single cell. This innovation could streamline the process, reduce contamination risks, and boost overall efficiency. Developing such microorganisms requires the introduction of cellulase genes, pentose metabolism pathways, and the WLP into host strains like yeast. However, challenges remain, such as mitigating the metabolic burden of expressing multiple pathways and ensuring the long-term genetic stability of engineered strains in tri-phase fermentation conditions.

Comprehensive TEAs and LCAs are critical for guiding TPF research toward industrial-scale implementation. Employing tools like SimaPro or GaBi, researchers can evaluate economic feasibility and environmental sustainability across various scenarios. Sensitivity analyses and uncertainty quantification will enhance the robustness of these assessments, while benchmarking TPF against existing bioethanol technologies will help highlight its competitive advantages and areas for improvement.

Further expanding the potential of TPF requires exploring novel feedstocks and diversifying its bioproducts. Utilizing agricultural residues, municipal solid waste, or industrial byproducts as feedstocks could broaden the applicability of TPF, provided adaptations are made to address specific compositional and pretreatment requirements. Beyond bioethanol, TPF could facilitate the production of platform chemicals like lactic acid, succinic acid, and 2,3-butanediol, or even bioplastics such as polyhydroxyalkanoates, enhancing its versatility and economic value.

Finally, continuous research into process intensification and optimization remains vital. Advanced reactor designs, such as microfluidic or rotating biofilm systems, could further improve mass transfer and overall efficiency. The integration of artificial intelligence and machine learning could

transform process control by predicting optimal fermentation conditions and enabling real-time adjustments to parameters, ensuring consistent and high-quality outputs.

11 Conclusions

The escalating global challenges of FW and the urgent need for renewable energy sources necessitate innovative and integrated approaches to address these pressing issues. The diverse characteristics of FW, encompassing its nutritional and lignocellulosic composition, as well as its variability across regions and sources, present both challenges and opportunities for its valorization. While conventional methods like anaerobic digestion and individual fermentation processes have shown promise, they also face limitations in terms of efficiency, substrate utilization, and environmental impact.

TPF, a novel and holistic approach integrating solid-state, liquid, and GF, emerges as a beacon of hope in this context. By leveraging the synergistic action of diverse microorganisms and emerging technologies like EH and supercritical water gasification, TPF has the potential to unlock the full potential of FW as a sustainable feedstock for bioethanol production. This study has demonstrated the feasibility of TPF through the integration of SSF, LF, and GF. Results include the SSF phase using enzymatic hydrolysis with *Aspergillus* species effectively breaking down the complex carbohydrates in FW, achieving sugar release up to 150 U/g TS of enzyme dosage. The successful conversion of these sugars to ethanol during the LF phase, with *Saccharomyces cerevisiae* achieving concentrations up to 10% (v/v) under optimized conditions. Furthermore, the potential of *Clostridium* in the GF phase to produce up to 0.62 g/L of ethanol and acetate from syngas using optimized conditions, generated from FW, further maximizing resource utilization.

Further research and development in areas such as strain engineering, process optimization, downstream processing, and techno-economic analysis are crucial to overcome existing challenges and pave the way for the industrial-scale implementation of this promising technology. Strategies for further development should focus on optimizing the integration of the three fermentation phases, exploring novel reactor designs, and developing cost-effective downstream processing techniques. Promoting research on TPF can be achieved through increased funding, interdisciplinary collaborations, knowledge dissemination, and the establishment of pilot-scale demonstration plants.

As we strive towards a circular bioeconomy, TPF exemplifies the transformative power of innovation and collaboration, showcasing the potential to not only convert FW into bioethanol but also to valorize the byproducts and residues generated at each stage, thus paving the way for a truly sustainable and resource-efficient future. Engaging policymakers and stakeholders is essential to create a supportive regulatory framework and to promote the adoption of TPF as a key technology for this transition.

Author Contributions

R.J.P.L.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. R.V.R., J.O., and A.S.: Conceptualization, Data curation, Investigation, Visualization, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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